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(54) Title: MALE FLOWER SPECIFIC GENE SEQUENCES (57) Abstract Three similar gene sequences are provided, the sequences being shown in the drawings, which are recovered from male flower parts of maize, specifically anther tissue. When one or more of these sequences are included in a gene construct, expression of an encoded protein is restricted to male parts of the plant. The sequences have utility in any application where expression in male flower parts is indicated, a specific application is in the control of expression of a disrupter protein which imparts male sterility when incorporated in a plant genome.		

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MALE FLOWER SPECIFIC GENE SEQUENCES

This invention relates to regulatory gene sequences which direct expression of a linked gene specifically to male parts of plants. The sequences to which the invention relates have utility as gene probes for locating male specific sequences in plants generally and is of particular utility in the development of male sterile plants for the production of F1 hybrid plants in situ.

By of general background, F1 hybrid plants are used extensively in most areas of agriculture because of their improved traits of one kind or another, such as increased yield, disease or low temperature resistance. F1 hybrids are produced by a manual process of emasculation of the intended female of the cross, to prevent self pollination, followed by application of pollen taken from the male of the cross to the female pollen receptors of the female of the cross. Maize, a major food crop, is almost exclusively planted as F1 hybrid plants. Maize carries its pollen producing parts as tassels at the terminal of the main stem with the female pollen receptors on quite separate structures in the lower parts of the plant. F1 hybrid production involved interplanting the two partners of the cross and growing to the stage when the tassels first appear. The tassels of the female member of the cross are then mechanically removed so that the

female are pollinated by the intended male which is allowed to mature and produce pollen.

The production of such hybrids is clearly labour intensive, which contributes greatly to the increased cost of hybrid seed. It is desirable that a new method be found to simplify the procedure and to reduce cost. One such possible procedure is the utilisation of inherently male sterile plants as the female parent of the cross. Cytoplasmic male sterility (CMS) has been used to advantage in hybrid seed production but the underlying cause of this type of sterility is not well understood and has in the past posed problems of disease such as the Southern corn leaf blight.

An object of the present invention is to provide a new approach to the production of F1 hybrids by manipulation of genes expressed only in the male parts of plants.

According to the present invention there are provided male flower specific cDNA sequences comprising the polynucleotides shown in Figures 4, 5 and 6 herewith, which are specifically expressed in male flower tissue.

The invention also provides the following:

Plasmid pMS10 in an Escherichia coli strain R1 host, containing the gene sequence shown in Figure 4 herewith, and deposited with the National Collection of Industrial & Marine Bacteria on 9th January 1989 under the Accession Number NCIB 40090.

Plasmid pMS14 in an Escherichia coli strain DH5 α host, containing the gene control sequence shown in Figure 5 herewith, and deposited with the National Collection of Industrial & Marine Bacteria on 9th January 1989 under the Accession Number NCIB

40099.

Plasmid pMS18 in an Escherichia coli strain
RI host, containing the gene control sequence shown
in Figure 6 herewith, and deposited with the
5 National Collection of Industrial & Marine Bacteria
on 9th January 1989 under the Accession Number NCIB
40100.

The isolation and characterisation of these
cDNA sequences and the utilisation of these cDNA
10 sequences as molecular probes to identify and
isolate the corresponding genomic sequences will
now be described.

The clones carrying the genomic sequences and
the preparation of a promoter cassette from one of
15 the clones illustrated using an approach and
techniques which may be equally applied to any of
the the clones. Furthermore the preparation of a
promoter fusion to a reporter gene and the
transformation of this construct into a test
20 species is described.

Unless stated otherwise, all nucleic acid
manipulations are done by standard procedures
described in Sambrook, Fritsch and Maniatis,
"Molecular Cloning: A Laboratory Manual", Second
25 Edition 1989.

The drawings which accomapny this application
show the following:

Figure 1 shows the library screening procedure used
for the isolation of maize flower specific clones;

30 Figure 2 shows dot blot analysis of total RNA
(4µg per dot) extracted from maize tassels of
increasing length.

Figure 3 A, B, C shows in situ hybridisation of
maize spikelet sections with pMS14 antisense RNA

probes.

Figure 4 shows the nucleotide and deduced amino acid sequence of MFS cDNA clone pMS10;

Figure 5 shows the nucleotide and deduced amino acid sequence of MFS cDNA clone pMS14;

Figure 6 shows the nucleotide and deduced amino acid sequence of MFS cDNA clone pMS18;

Figure 7 is a restriction map of the 9kb EcoRI fragment from clone 10/CT8-3;

Figure 8 is a restriction map of the 9kb EcoRI fragment from clone 14/17M;

Figure 9 is a restriction map of the 9kb EcoRI fragment from clone 18/CT3;

Figure 10 is a plasmid map of clone pMS10-5;

Figure 11 shows the structure of pTAK1, pTAK2 and pTAK3; and,

Figure 12 is a map of clone pMS10-6GUS.

EXAMPLE 1

1. Isolation and Characterisation of Male Flower Specific cDNA from Maize

To clone cDNAs to genes which are expressed in the male flowers of maize we constructed two cDNA libraries. In maize, the male flowers are born in the tassel which terminates the main stem. Library 1 was prepared from poly [A] RNA from whole maize tassels bearing early meiotic anthers (most meiocytes in early meiotic prophase) and library 2 from poly [A]⁺ RNA from whole tassels bearing late meiotic anthers (predominantly diad and early tetrad stages). Figure 1 reviews the library screening procedure used and this yielded five unique early meiotic MFS cDNAs and one unique late meiotic cDNA. Clone PMS3, a partial cDNA of 120 base pairs, isolated by the differential screening

process, was subsequently used as a hybridisation probe to isolate the corresponding pending near full-length clone, PMS18.

Table 1 below summarises some of the features of each of these cDNA clones. Expression of the mRNAs of the five MFS cDNAs isolated from the early meiotic library is detected in RNA isolated from both early and late meiotic tassel samples. The mRNAs corresponding to these cDNAs are not wholly specific to male flowers and are detected at considerably lower levels in leaves (pMS10 and pMS18) or in leaves, cobs and roots (pMS1, pMS2 and pMS4) Table 1. In contrast pMS14 mRNA is found only in late meiotic RNA and is not detected in leaves, cobs or roots (Table 1).

<u>TABLE 1</u>						
	pMS1	pMS2	pMS4	pMS10	pMS14	pMS18
Library ¹	1	1	1	1	2	1
Insert size ²	750	500	720	1350	620	940
mRNA size ³	900	950	850	1600	900	1100
Organ specificity ⁴	+	+	+	++	+++	++
Expression window ⁵	E/L	E/L	E/L	E/L	L	E/L

Table Legend

- 1 Isolated from cDNA library 1 (early meiotic)
or library 2 (late meiotic).
- 2 Approximate size in base pairs.
- 5 3 Approximate size in nucleotides.
- 4 + = expressed in tassels and at much lower
levels in leaves, cobs and roots.
++ = expressed in tassels only and at much
lower levels in leaves.
- 10 +++ = expressed in tassels only.
- 5 E/L = mRNA present in RNA from both early and
late meiotic tassels.
L = mRNA present only in RNA from late meiotic
tassels.
- 15 We have examined expression of the genes
corresponding to these cDNAs during tassel
development using dot blot hybridisations (Figure
2). The dot blot analysis was generated by binding
total; RNA to nitrocellulose followed by
- 20 hybridisation to radiolabelled pMS cDNAs. All
filters were exposed to film for 48 hours at -70°C
except pMS10 which was exposed for 168 hours. The
tassel lengths in each sample were as follows: A ≥
2cm; B=2-5cm; C=5-10cm; D=10-15cm; E= 15-20cm;
- 25 F=20-30cm; and G=20-30cm. The solid bars in Figure
2 show the developmental stage relative to
microsporogenesis in each of the samples: PM =
premeiosis; M = meiosis; IP = immature pollen; and
MP = mature pollen.
- 30 The early meiotic mRNAs (pMS1, 2, 4, 10 and
18) accumulate very early in development in tassels
less than 2 cm in length. We have not analysed
expression in floral meristems prior to this stage.
These mRNAs persist through the meiotic anther

stages and then decline as pollen grains mature. In contrast the late meiotic mRNA of pMS14 is not detected in tassels less than 5 cm in length, but increases dramatically as the sporogenous cells of the anther enter meiosis (Figure 2). As with the early meiotic mRNAs, pMS14 mRNA declines abruptly as mature pollen accumulates in the anthers (Figure 2).

These data show that different temporal controls of gene expression occur during development of male flowers in maize. The controls which programme accumulation of the early meiotic mRNAs are probably very similar but contrast markedly with those regulating appearance and accumulation of the late meiotic mRNA, pMS14. Both the early and late meiotic mRNAs are involved with developmental processes which occur prior to the accumulation of mature pollen grains. They are clearly not involved with the later stages of anther development such as dehiscence nor are they mRNAs which accumulate in mature pollen.

The technique of in situ hybridisation has been used to determine the tissue localisation of MFs mRNAs in male flowers of maize. The techniques used are described in Wright and Greenland (1990; SEB Seminar Series, vol 43 ed by N Harris and D Wilkman. Cambridge University Press, Cambridge; in the Press). The data shown is that for pMS14 mRNA.

Figure 3 A,B shows in situ hybridisation with pMS14 antisense RNA probes. Sense and antisense probes more prepared by sub cloning a 300 basic pair fragment of pMS14 into the vector, pBS, followed by preparation of radiolabelled T3 and T7 polymerise transcripts utilising methods suggested

by the supplier of the vector (Stratagene, Trade Mark). These hybridisations show that pMS14 mRNA is located in the tapetal cell layer surrounding the developing microspores. Hybridisation of the pMS14 antisense probe does not occur to any other cells in the section. Likewise the pMS14 sense probe does not show any specific hybridisation (Figure 3c). These sections were made from 15-20 cm maize tassels at a stage when the level of pMS14 mRNA is at a maximum (Figure 2). In these sections and in those from subsequent experiments hybridisation occurs to the tatum of the anthers in one floret but not the other. In Figure 3 A,B the tapetal layers which contain pMS14 mRNA surround late meiotic microspores at the tetrad stage whilst the tapetal layers not containing pMS14 mRNA surround sporogenous cells which have not undergone meiosis. It is a feature of maize that the sets of anthers within the individual florets of the spikelet do not develop co-ordinately. Thus in situ hybridisation shows that accumulation of pMS14 mRNA is tissue-specific and confirm data obtained from dot blot analysis (Figure 2) that expression of PMS14 mRNA is stage specific as it is first detected in tapetum surrounding meiotic cells.

EXAMPLE 2

Determination of DNA sequence of pMS10

DNA from cDNA clone, pMS10, for sequence analysis by subcloning into M13mp18 using standard procedures. The nucleotide sequences of the subclones were determined by the dideoxy method using standard procedures. In addition a Sequence (Trade Mark) method was used utilising methods

described by the suppliers. Regions of the clones were sequenced by priming with synthetic oligonucleotides synthesised from sequence obtained from previous gel readings. Oligonucleotide concentrations used for priming were identical to those used with universal primers.

MFS, Clone pMS10 full length cDNA of 1353 base pairs. The complete nucleotide sequence and the predicted amino acid sequence are shown in Figure 4. The sequence contains an open reading frame of 1022 nucleotides encoding a polypeptide of 341 amino acids with a deduced molecular weight of 37371 kd the polypeptide is rich in glycine residues. The open reading frame is flanked by 5' and 3' non-translated regions of 129 and 201 bases respectively.

EXAMPLE 3

Determination of DNA sequence of pMS14

Procedure of determining nucleotide sequence as described in Example 2.

Clone pMS14 is an in complete cDNA of 581 base pairs the complete nucleotide sequence and deduced amino acid sequence are shown in Figure 5. The sequence contains an open reading frame which extends from nucleotide 1 to 278 encoding a partial polypeptide of 127 amino acids. The polypeptide is particularly rich in alanine and arginine residues. The open reading frame is flanked by 3' non-coding region 203 nucleotides. A consensus processing and polyadenylation signal hexanucleotide, AATAAA occurs at position 548.

EXAMPLE 4

Determination of DNA sequence of pMS18

Procedure for determining nucleotide sequence

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as described in Example 2.

Clone pMS18 is a near full-length cDNA of 933 bases. The complete nucleotide sequence and deduced amino acid sequence is shown in Figure 6. pMS18 lacks 28 nucleotides at its 3' terminus. The missing nucleotides are present in clone pMJ3 which overlaps the sequence of pMS18 by a further 91 nucleotides. pMS3 was the original clone isolated by differential screening of cDNA inbranes and was subsequently used as a hybridisation probe to isolate pMS18. pMS18 contains an open reading frame extending from nucleotide 151 to 813 and encodes a polypeptide of 221 amino acids with a deduced molecular weight of 25 kilodartons. The polypeptide is particularly rich in arginime residues. The open reading is flanked by 5' and 3' non-coding regions of 150 and 120 nucleotides respectively.

EXAMPLE 5

Isolation of genomic clones corresponding to pMS10

Genomic DNA clones carrying genes corresponding to the cDNA, pMS10 were isolated from an EMBL 3 phase library of partial Mb01 fragments of maize DNA. The library was screened using radiolabelled "long-mer" probes synthesised in an in vitro labelling system. This system comprised, 50 mg of a synthetic 100 base oligonucleotide (base position 452-551 at pMS10; Figure 4). 500 mg of a synthetic primer oligonucleotide, sequence - TAGTTTCCT-CCGCTAG and which will base pair with the 3' end of the long olionucleotide, one or two radiolabelled oligonucleotides (usually ³²PdCTP and/or ³²P-dGTP) and 5-10 units of the Klenow

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fragment of DNA polymerase 1. The reactions were performed at 37°C for 30 minutes in a buffer identical to that used for the "random-priming" method of DNA labelling except that the random hexanucleotides were omitted. Five million phase clones immobilised on nylon "Hybaid" (Trade Mark) filters were hybridised at 65°C with these probes using prehybridisation and hybridisation buffers suggested by the suppliers of the filters (Amersham International). Filters were washed on 3 x SSC, 0.1 % SDS at 65°C using these procedures 50-60 EMBL3 phage clones containing either complete or partial regions of a pMS10 gene were obtained. The DNA from three EMBL3 phage clones 10/CT8-1, 10/CT8-3 and 10/CT25-3 which combined complete pMS10 genes was prepared and analysed by restriction enzyme digests. Each of these clones was shown to contain a common 9Kb EcoRI fragment which extends from the third intron of the pMS10 gene into the 5' non-coding and promoter regions of the gene. A partial restriction map of the 9 Kb EcoRI fragment is shown in Figure 7.

EXAMPLE 6

Isolation of genomic clones corresponding to pMS14

To isolate genomic DNA clones carrying genes corresponding to the cDNA, pMS14 two approaches were taken. In the first approach the method shown in Example 5 was adopted except the 5 million phage clones were screened with the complete cDNA sequence and the wash stringencies after hybridisation procedure yielded two positive clones 14/CTA and 14/CTD. In the second approach a 12 Kb EcoRI cut fraction of maize genomic DNA, shown by Southern Blotting to carry the pMS14 gene, was

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ligated into EcoRI cut λ phage EMBL4 DNA to produce a library of cloned 17 Kb DNA fragments. Roughly 200,000 clones were screened as described above, and two positive clones, 14/17m and 14/17R which combined a 17 Kb EcoRI fragment which hybridized to pMS14, were isolated. On further analysis the two positive clones isolated from the partial MboI/EMBL3 library were found to contain an internal 17 Kb fragment. A partial restriction map of this 17 Kb EcoRI fragment, common to all the clones, is shown in Figure 8.

EXAMPLE 7

Isolation of genomic clones corresponding to pMS18

To isolate genomic DNA clones carrying genes corresponding to the cDNA pMS18, the procedure described in Example 5 was adopted. Five million EMBL3 phage clones were hybridized to a "long-mer" probe derived from the sequence of pMS18, position 133-222 (Figure 6). The sequence of the 3' complementary oligonucleotide was a 5'-GCCTCGGCGGTCGAC-3'. Two clones, 18/CT3 and 18/CT23, carrying the pMS18 gene were isolated from this screen. Restriction mapping of these clones showed that they both contained a 4.5 Kb BamHI-SalI fragment comprising the 5' region of the coding sequence of pMS18 and approximately 4 Kb of the promoter and upstream region of the gene. A partial restriction map of clone 18/CT3 is shown in Figure 9.

EXAMPLE 8

Construction of a promoter cassette derived from 10/CT8-3

The following subclones from the λ EMBL3 clone 10/CT8-3 were made. The 4.5 Kb PstI-EcoRI fragment

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was cloned into pUC18 to give pMS10-2. The 2.7 Kb XbaI-EcoRI fragment was cloned into pUC 18 to give pMS10-3. The 1.6 Kb HindIII to XbaI fragment was cloned into pUC18 to give pMS10-4.

5 The polymerase chain reaction (PCR) was used to amplify a 930 bp fragment from pMS10-3. The primers used for the PCR reaction were as follows. Primer pUC/2 is homologous to pUC sequence flanking the polylinker site. Primer 10/9 is complementary
10 to the sequence of pMS10 from position 106-129 except that it contains an additional thymidine residue between bases 123 and 124. The sequence of these primers is:

pUC/2 5' CGACGTTGTAAAACGACGGCCAGT-3'

15 10/9 5' AGTCGGATCCCCCCCCGCGCAGCCG-3'

Following amplification in the PCR reaction a DNA fragment is produced in which the flanking XbaI site and the sequence identical to that present in the corresponding region of clone 10/CT8-3 up to the base immediately prior to the translation
20 initiator are faithfully reproduced except that a novel BamHI site is introduced by the introduction of the thymidine residue. This 930 bp fragment was gel purified, and digested with XbaI and BamHI. It
25 was then cloned into pMS10-4 which had been previously digested with XbaI and BamHI to yield clone pMS10-5. In pMS10-5 the sequences required for promoter activity associated with the MS10 gene are reacted and modified such that the promoter can
30 now be fused to any gene via the BamHI site which occurs immediately prior to the translation start point. That these and no other modifications had occurred was confirmed by sequence analysis.

EXAMPLE 9Construction of a promoter fusion between Msl0 gene and the glucuronidase reporter gene

- 5 The 1830 bp HindIII to BamHI fragment from pMS10-5 was ligated into pTAK1, previously cut with HmdIII and Bam HI. pTAK1 is based on the binary plant transformation vector Bin 19 (Bevan, 1984; Nucleic Acids Research 12, 8711) and carries the glucuronidase (GUS) reporter gene and Nos 3' terminator (Figure 11). The resulting plasmid was termed pMS10-6GUS and makes a transcriptional gene fusion between the promoter of the MS10 gene and the GUS reporter gene.

EXAMPLE 10

- 15 Transformation of tobacco plants with MS10 promoter gene constructs

- The recombinant vector pMS10-6GUS as mobilised from E. Coli (TG-2) onto Agrobacterium tumefaciens (LBA4404) in a triparental mating on L-plates with
20 E Coli (HB101) harbouring pRK2013. Transconjugants were selected on minimal medium containing kanamycin ($50\mu\text{g}/\text{cm}^3$) and streptomycin ($500\mu\text{g}/\text{cm}^3$).

- L-Broth (5 cm^3) containing kanamycin at $50\text{ g}/\text{cm}^3$ was inoculated with a single Agrobacterium
25 colony. The culture was grown overnight at 30°C with shaking at 150 rpm. This culture ($500\mu\text{l}$) was inoculated into L-Broth containing kanamycin ($50\mu\text{ g}/\text{cm}^3$) and grown as before. Immediately before use the Agrobacteria were pelleted by spinning at 3000
30 rpm for 5 minutes and suspended in an equal volume of liquid Murashige and Skoog (MS) medium.

Feeder plates were prepared in 9 cm diameter petri dishes as follows. Solid MS medium supplemented with 6-benzyl-aminopurine (6-BAP) (1

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mg/l) and 1-naphthaleneacetic acid (NAA) (0.1 mg/l) was overlaid with Nicotiana tabacum var Samsun suspension culture (1 cm^3). One 9 cm and one 7cm filter paper discs were placed on the surface.

Whole leaves from tissue culture grown plants were placed in the feeder plates. The plates were sealed with "Nescofilm" (Trade Mark) and incubated overnight in a plant growth room (26°C under bright fluorescent light).

Leaves from the feeder plates were placed in Agrobacteria suspension in 12 cm diameter petri dishes and cut into 1- 1.5 cm^2 sections. After 20 minutes the leaf pieces were returned to the feeder plates which were sealed and replaced in the growth room. After 48 hours incubation in the growth room the plant material was transferred to MS medium supplemented with 6-BAP (1 mg/l), NAA (0.1 mg/l), carbenicillin ($500\mu\text{g}/\text{cm}^3$) and kanamycin ($100\mu\text{g}/\text{cm}^3$), in petri dishes. The petri dishes were sealed and returned to the growth room.

Beginning three weeks after inoculation with Agrobacterium, shoots were removed from the explants and placed on MS medium supplemented with carbenicillin ($200\mu\text{g}/\text{cm}^3$) and kanamycin ($100\mu\text{g}/\text{cm}^3$) for rooting. Transformed plants rooted 1-2 weeks after transfer.

Following rooting, transformed plants were transferred to pots containing soil and grown in the glasshouse. Roughly one month after transfer the plants flowered.

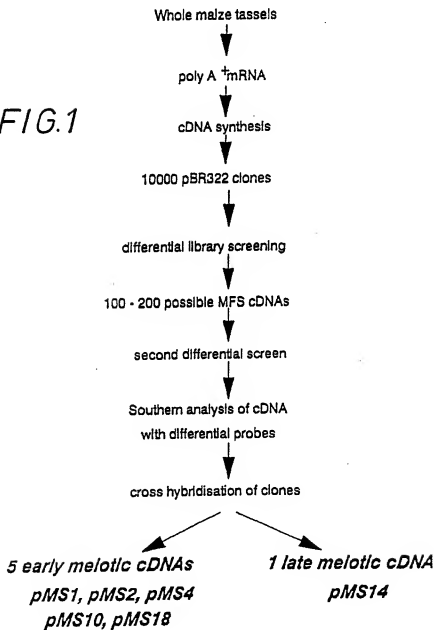
The anthers of the tobacco plants containing the pMS10-6GUS construct were sprayed for GUS activity using standard procedures.

1. A male flower specific cDNA sequence comprising the polynucleotide shown in Figure 4 herewith, which is specifically expressed in male flower tissue and variants therein
5 permitted by the degeneracy of the genetic code.
2. A male flower specific cDNA sequence comprising the polynucleotide shown in Figure 5 herewith, which is specifically expressed in male flower tissue and variants therein
5 permitted by the degeneracy of the genetic code.
3. A male flower specific cDNA sequence comprising the polynucleotide shown in Figure 6 herewith, which is specifically expressed in male flower tissue and variants therein
5 permitted by the degeneracy of the genetic code.
4. Plasmid pMS10 in an Escherichia coli strain R1 host, containing the gene sequence shown in Figure 4 herewith, and deposited with the National Collection of Industrial & Marine Bacteria on 9th January 1989 under the
5 Accession Number NCIB 40090.

5. Plasmid pMS14 in an Escherichia coli
strain DH5 α host, containing the gene control
sequence shown in Figure 5 herewith, and
deposited with the National Collection of
5 Industrial & Marine Bacteria on 9th January
1989 under the Accession Number NCIB 40099.

6. Plasmid pMS18 in an Escherichia coli
strain R1 host, containing the gene control
sequence shown in Figure 6 herewith, and
deposited with the National Collection of
5 Industrial & Marine Bacteria on 9th January
1989 under the Accession Number NCIB 40100.

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ISOLATION OF cDNA CLONES**FIG.1****SUBSTITUTE SHEET**

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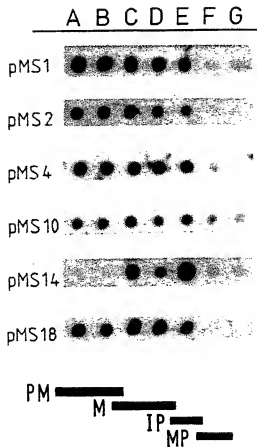


FIG. 2.

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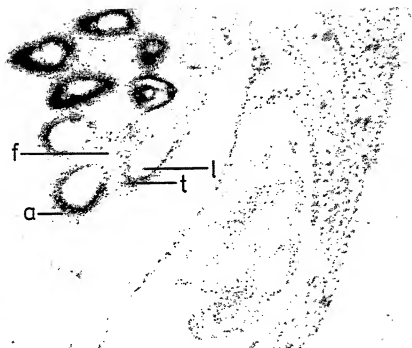
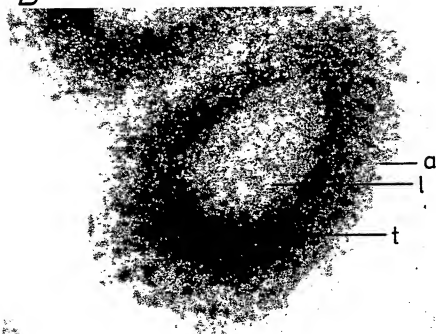


FIG. 3A.

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B*FIG. 3B.**C**FIG. 3C.*

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FIG. 4.

Nucleotide and deduced amino acid sequence for male flower specific cDNA clone, pMS10.

	10		20		30		40		
GCG	CTT	GCC	CGC	TCG	TTC	CCC	TCG	CCT	CCC
							CGG	TCG	CGC
								CGC	TCC
	50		60		70		80		90
CGC	TGC	CGC	CGT	GGC	GAT	TCC	TGC	CCG	GCG
							GCG	GCG	CCG
								GGT	TCA
	100		110		120		130		
GGT	CCA	CGG	CGG	CGG	CTG	CGC	GGG	GCG	GGA
							CCG	ACT	ATG
								Met	Gly
	140		150		160		170		180
CGG	ACG	ACA	GCA	AGA	TCT	CCC	CCG	ACG	AGG
Arg	Thr	Thr	Ala	Arg	Ser	Pro	Pro	Thr	Arg
								Asn	Ser
								Phe	Glu
								Gly	
	190		200		210		220		
TGC	GAC	TAC	AAC	CAC	TGG	CTC	ATC	ACC	ATG
Cys	Asp	Tyr	Asn	His	Trp	Leu	Ile	Thr	Met
								Asp	Phe
								Pro	Asp
								Pro	
	230		240		250		260		270
AAG	CCG	TCG	CGC	GAA	GAG	ATG	ATC	GAG	ACA
Lys	Pro	Ser	Arg	Glu	Glu	Met	Ile	Glu	Thr
								Tyr	Leu
								Gln	Thr
								Leu	
	280		290		300		310		
GCC	AAG	GTC	GTC	GGG	AGT	TAT	GAG	GAG	GCC
Ala	Lys	Val	Val	Gly	Ser	Tyr	Glu	Glu	Ala
							Lys	Lys	Arg
								Met	Tyr
	320		330		340		350		360
GCT	TTT	AGT	ACG	ACG	ACT	TAT	GTT	GGT	TTT
Ala	Phe	Ser	Thr	Thr	Thr	Tyr	Val	Gly	Phe
									CAG
									GCT
									GTA
									ATG
									ACC
	370		380		390		400		
GAG	GAA	ATG	TCA	GAA	AAA	TTT	CGC	GGT	TTG
Glu	Glu	Met	Ser	Glu	Lys	Phe	Arg	Gly	Leu
									Pro
									Gly
									GTA
									Val
									Val
									Phe
	410		420		430		440		450
ATT	TTG	CCT	GAT	TCA	TAT	CTA	TAT	CCA	GAA
Ile	Leu	Pro	Asp	Ser	Tyr	Leu	Tyr	Pro	Glu
									Thr
									Lys
									Glu
									Tyr
									Gly
	460		470		480		490		
GGA	GAC	AAA	TAT	GAC	AAT	GGT	GTC	ATC	ACT
Gly	Asp	Lys	Tyr	Asp	Asn	Gly	Val	Ile	Thr
									Pro
									Arg
									Pro
									Pro
									Pro

FIG. 4.

(cont.)

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500	510	520	530	540
GTT CAT TAT AGC AGA CCA TCA AGA ACT GAC AGG AAC CGT AAC TAC				
Val His Tyr Ser Arg Pro Ser Arg Thr Asp Arg Asn Arg Asn Tyr				
550	560	570	580	
CGA GGA AAC TAC CAG GAT GGC CCT CCA CAG CAA GGA AAT TAC CAG				
Arg Gly Asn Tyr Gln Asp Gly Pro Gln Gln Gly Asn Tyr Gln				
590	600	610	620	630
AAC AAC CGT CCT CCA CCA GAA GGT GGT TAC CAG AAC AAC CCG CCG				
Asn Asn Arg Pro Pro Glu Gly Gly Tyr Gln Asn Asn Pro Pro				
640	650	660	670	
CAG CAA GGA AAC TAC CAG ACA TAC CGC TCG CAG CAA GAT GGA AGA				
Gln Gln Gly Asn Tyr Gln Thr Tyr Arg Ser Gln Gln Asp Gly Arg				
680	690	700	710	720
GGC TAT GCC CCA CAG CAG AAT TAT GCA CAA GGT GGT CAG GAT GGT				
Gly Tyr Ala Pro Gln Gln Asn Tyr Ala Gln Gly Gly Gln Asp Gly				
730	740	750	760	
AGA GGT TTT GGA AGG AAT GAT TAC ACA GAC CGT TCA GGC TAC AAT				
Arg Gly Phe Gly Arg Asn Asp Tyr Thr Asp Arg Ser Gly Tyr Asn				
770	780	790	800	810
GGA CCC ACT GAT TTT CGA AGT CAA ACT CAG TAC CAA GGG CAT GTA				
Gly Pro Thr Asp Phe Arg Ser Gln Thr Gln Tyr Gln Gly His Val				
820	830	840	850	
AAT CCA GCT GGG CAA GGT CAA GGT TAC AAC AAC CCC CAA GAG CGT				
Asn Pro Ala Gly Gln Gly Gln Gly Tyr Asn Asn Pro Gln Glu Arg				
860	870	880	890	900
ACG AAC TTC TCG CAA GGG CAG GGA GGA GGT TTT AGG CCT GGT GGT				
Thr Asn Phe Ser Gln Gly Gln Gly Gly Gly Phe Arg Pro Gly Gly				
910	920	930	940	
CCT TCA GCA CCT GGG TCT TAT GGC CAA CCA TCA GCA CCT GGA TCT				
Pro Ser Ala Pro Gly Ser Tyr Gly Gln Pro Ser Ala Pro Gly Ser				
950	960	970	980	990
TAT GGT CAA CCT AAT ACA CTT GGT AAC TAT GGG CAG GTA CCT CCA				
Tyr Gly Gln Pro Asn Thr Leu Gly Asn Tyr Gly Gln Val Pro Pro				

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FIG. 4.

(cont.)

	1000		1010		1020		1030							
TCA	GTG	AAT	CCT	GGT	GGT	AAC	AGA	GTT	CCT	GGT	GTG	AAT	CCT	AGT
Ser	Val	Asn	Pro	Gly	Gly	Asn	Arg	Val	Pro	Gly	Val	Asn	Pro	Ser
	1040		1050		1060		1070		1080					
TAT	GGT	GGG	GAT	GGC	AGA	CAG	GGG	GCT	GGA	CCA	GCA	TAT	GGT	GGA
Tyr	Gly	Gly	Asp	Gly	Arg	Gln	Gly	Ala	Gly	Pro	Ala	Tyr	Gly	Gly
	1090		1100		1110		1120							
GAT	AAC	TGG	CAA	AGA	GGT	TCT	GGT	CAG	TAT	CCT	AGC	CCA	GGT	GAA
Asp	Asn	Trp	Gln	Arg	Gly	Ser	Gly	Gln	Tyr	Pro	Ser	Pro	Gly	Glu
	1130		1140		1150		1160		1170					
GGA	CAA	GGA	AAC	TGG	CAG	GGA	AGG	CAG	TAA	GAG	CTG	ACG	TGT	TCC
Gly	Gln	Gly	Asn	Trp	Gln	Gly	Arg	Gln						
	1180		1190		1200		1210							
ACT	GAA	GAC	AAG	AAT	GGC	ACT	TGA	GAT	TTA	GAA	ATC	TCC	ATC	TGT
	1220		1230		1240		1250		1260					
AAA	ATA	AAC	GAC	TGT	GAT	GCA	TTA	CTC	TTT	TTT	TTT	TTC	TTG	CAT
	1270		1280		1290		1300							
TTG	AAC	TCT	AAA	CTT	ATG	GGC	ATG	CGT	TAT	TAC	CAA	ACT	ACG	GAT
	1310		1320		1330		1340		1350					
GCA	AAT	TCA	TTT	TAG	TTT	TTT	GGG	CCA	AAT	GTT	GGC	ATT	TTT	AAA

AAA

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FIG.5.

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Nucleotide and deduced amino acid sequence for the male flower specific cDNA clone, pMS14.

	10		20		30		40							
GCA	GGG	GGG	GGG	GCA	CAG	CAA	GCC	AGC	AGA	GCA	GAA	AGC	AGC	CGC
Ala	Gly	Gly	Gly	Ala	Gln	Gln	Ala	Ser	Arg	Ala	Glu	Ser	Ser	Arg
	50		60		70		80		90					
AGC	CCC	AGC	CCC	CAC	AAA	GAC	GAA	GGC	AAC	AAT	GGC	GCT	AGA	AGC
Ser	Pro	Ser	Pro	His	Lys	Asp	Glu	Gly	Asn	Asn	Gly	Ala	Arg	Ser
	100		110		120		130							
AGC	CAC	GCC	CCC	CGC	GCA	CTC	CTC	GCG	CGT	GCC	TCG	TCC	TGC	TGG
Ser	His	Ala	Pro	Arg	Ala	Leu	Leu	Ala	Arg	Ala	Ser	Ser	Cys	Trp
	140		150		160		170		180					
TCC	TCG	GCG	GCG	GCA	CCG	GCC	CGT	CGT	CGG	TGC	TCA	GCG	CGC	CGG
Ser	Ser	Ala	Ala	Ala	Pro	Ala	Arg	Arg	Arg	Cys	Ser	Ala	Arg	Arg
	190		200		210		220							
GGC	GCA	GGA	CCG	GCG	GCA	GTG	CCT	GCC	GCA	GCT	GAA	CGC	CTC	CTG
Gly	Ala	Gly	Pro	Ala	Ala	Val	Pro	Ala	Ala	Ala	Glu	Arg	Leu	Leu
	230		240		250		260		270					
CGG	TGC	CGC	GCG	TAC	CTG	GTG	CCG	GCG	CGC	CGG	ACC	CCA	GCG	CGG
Arg	Cys	Arg	Ala	Tyr	Leu	Val	Pro	Ala	Arg	Arg	Thr	Pro	Ala	Arg
	280		290		300		310							
ACT	GCT	GCA	GCG	CTG	ACG	CGC	CGT	GTG	CAC	GAG	TGC	GCC	TGC	AGC
Thr	Ala	Ala	Ala	Leu	Thr	Arg	Arg	Val	His	Glu	Cys	Ala	Cys	Ser
	320		330		340		350		360					
ACC	ATG	GGC	ATC	ATC	AAC	AGC	CTG	CCC	GGC	CGG	TGC	CAC	CTC	GCC
Thr	Met	Gly	Ile	Ile	Asn	Ser	Leu	Pro	Gly	Arg	Cys	His	Leu	Ala
	370		380		390		400							
CAA	GCC	AAC	TGC	TCC	GCT	TGA	AGC	AGG	GAC	CTG	GCA	CGC	GTG	CTG
Gln	Ala	Asn	Cys	Ser	Ala									
	410		420		430		440		450					
CAA	TGG	ATG	GCA	GGA	GGG	GAG	AGG	AAT	AAG	AAG	TGT	TTC	CAT	TTC
	460		470		480		490							
ACA	GTG	AGA	GCA	GTC	GAG	CTC	CAA	CGT	TGT	CGT	CGT	CGT	CGT	CTT

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FIG. 5.

(cont.)

500		510		520		530		540
CTT	CTT	TTG	ATA	TTC	AGA	CTC	TGT	CTT
							GCG	GTC
							TAT	ATC
							ATC	AGC
		550		560		570		580
ATA	ATA	ATA	ATA	AAA	TAA	GTA	AAA	CCA
							AAA	AAA
							AAA	AAA
							AA	

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FIG. 6.

(cont.)

500	510	520	530	540
CGC CGG CTC CGT CCC CGA GCA CGC CAA CAA GCC CTG AAC GCC AAC				
Arg Arg Leu Arg Pro Arg Ala Arg Gln Gln Ala Leu Asn Ala Asn				
550	560	570	580	
AAG CGT GGT AGT AGA GGT GCT ACT GTT ACT GTA GTA CGT CGT CGT				
Lys Arg Gly Ser Arg Gly Ala Thr Val Thr Val Val Arg Arg Arg				
590	600	610	620	630
CTT CAT GCA TGC GTG GTT CGT GGT TTC CCT AGC TCC ATA CGA GCA				
Leu His Ala Cys Val Val Arg Gly Phe Pro Ser Ser Ile Arg Ala				
640	650	660	670	
GTA GTT GGG CTT GCA CGT ACC GTA CGT CTA GCT AGC TAT ATA TAT				
Val Val Gly Leu Ala Arg Thr Val Arg Leu Ala Ser Tyr Ile Tyr				
680	690	700	710	720
GCT TGT GTT CTA CTG CTT TTT AGT TTA ATT ACC TGC CTG CAT TGG				
Ala Cys Val Leu Leu Leu Phe Ser Leu Ile Thr Cys Leu His Trp				
730	740	750	760	
AGA GTT GGA TCT GTT TCA TTT GGT GGT GTT TGC TTT ACT ATT AGG				
Arg Val Gly Ser Val Ser Phe Gly Gly Val Cys Phe Thr Ile Arg				
770	780	790	800	810
TCA GTA TCT GTT TGT GGA GAC TTG GTG TTT AAT TTA TTT AGC CGT				
Ser Val Ser Val Cys Gly Asp Leu Val Phe Asn Leu Phe Ser Arg				
820	830	840	850	
TTG TGA CTG GTT GTA GCT AGC GGT GGT GCG GTG GTG ATG TTC TTG				
Leu				
860	870	880	890	900
AGG CAT GAA TAA TGC TAC ATG CAT GTG ATG TAT CCA TGT TTT GTG				
910	920	930		
TGT GGT AAA CCT GTT GTT TGT ATA AGC TGT CCC				

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Restriction map of 9 kb EcoRI fragment from clone 10/CT8-3

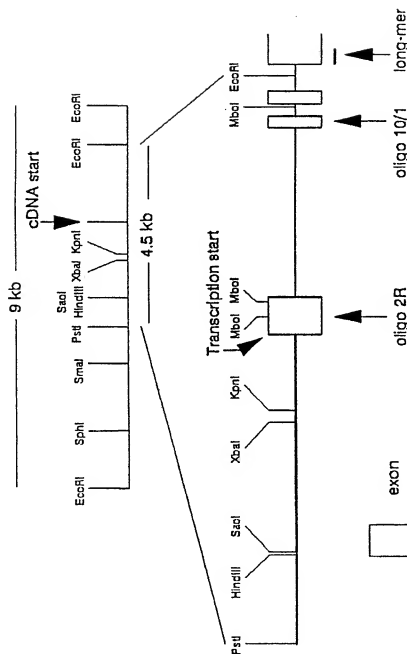


FIG. 7.

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Restriction map of 17 kb EcoRI fragment from clone 14/17M

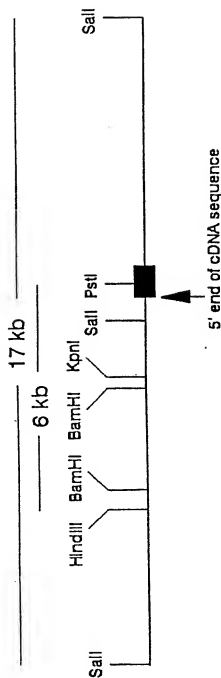


FIG. 8.

Restriction map of 16 kb EcoRI fragment from clone 18/CT3

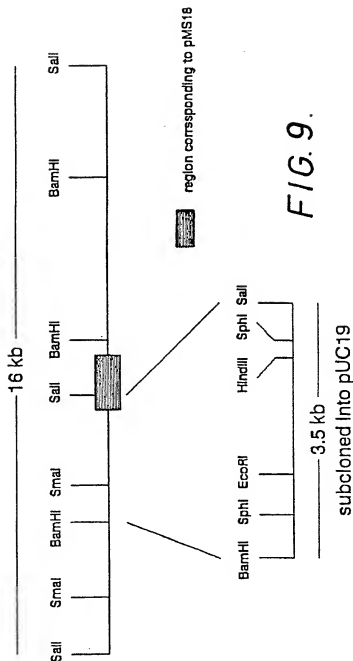


FIG. 9.

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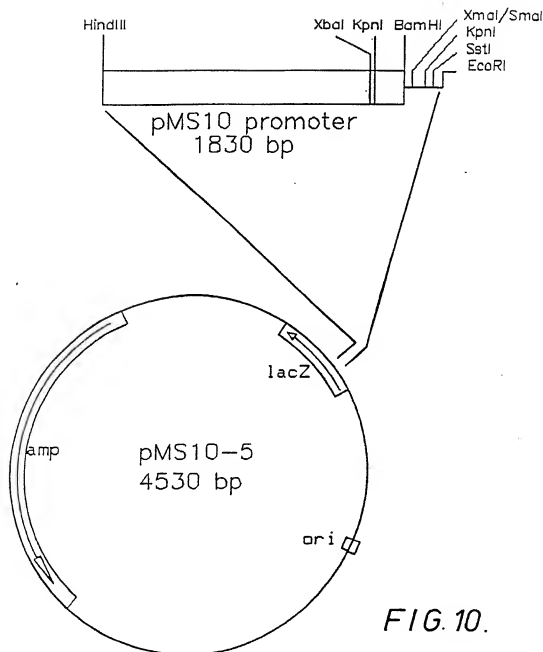


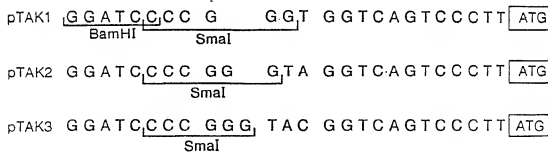
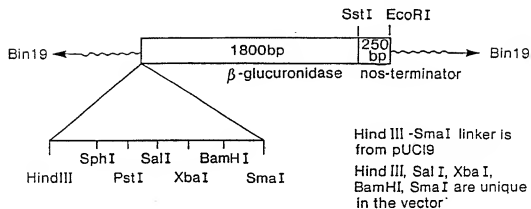
FIG. 10.

Clone pMS10-5.

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FIG. 11.

Structure of pTAK1, pTAK2, pTAK3



Map of clone pMS10-6GUS

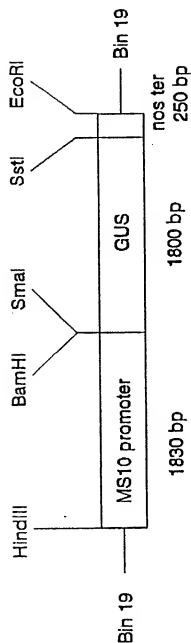


FIG. 12.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 90/00111

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC⁵: C 12 N 15/29

II. FIELDS SEARCHED

Minimum Documentation Searched

Classification System

Classification Symbols

IPC⁵

C 12 N

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched

III. DOCUMENTS CONSIDERED TO BE RELEVANT*

Category ¹	Citation of Document, ¹¹ with Indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
O,A	Journal of Cellular Biochemistry, Supplement 12C, UCLA Symposium on the Molecular Basis of Plant Development, 26 March - 2 April 1988, Alan R. Liss, Inc., (New York, US), A.J. Greenland et al.: "Isolation and characterisation of developmentally expressed genes from maize tassels", page 171, abstract L 208 see the abstract --	1-6
O,A	UCLA Symp. Mol. Cell. Biol., New Ser., volume 92 (Mol. Basis Plant Dev.), 1989, Alan R. Liss, Inc., J.P. Mascarenhas: "Characterization of genes that are expressed in pollen", pages 99-105 & Proceedings of an E.I. du Pont de Nemours-UCLA Symposium, Steamboat Springs, Colorado, 26 March - 2 April 1988 see the whole document -- ./.	1-6

* Special categories of cited documents: ¹⁰

"A" document defining the general state of the art which is not
considered to be of particular relevance

"E" earlier document but published on or after the international
filing date

"L" document which may throw doubts on priority claim(s) or
which is cited to establish the publication date of another
claim or other special reason (see specification)

"O" document referring to an oral disclosure, use, exhibition or
other means

"P" document published prior to the international filing date but
later than the priority date claimed

"T" later document published after the international filing date
or priority date and not in conflict with the application but
cited to understand the principle or theory underlying the
invention

"X" document of particular relevance: the claimed invention
cannot be considered novel or cannot be considered to
involve an inventive step

"Y" document of particular relevance: the claimed invention
cannot be considered to involve an inventive step when the
document is combined with one or more other such docu-
ments, such combination being obvious to a person skilled
in the art

"Z" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

7th may 1990

International Searching Authority

EUROPEAN PATENT OFFICE

Date of Mailing of this International Search Report

08 JUN 1990

Signature of Authorized Officer

MISS T. TAZHAR

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	Biological Abstracts/RRM, BR36:27774, J.P. Mascarenhas: "Anther and pollen- expressed genes", see the abstract, & Plant Gene Research: Basic Knowledge and Application: Temporal and Spatial Regulation of Plant Genes. XIII+344P. Springer-Verlag: Vienna, Austria; New York, New York, USA. Illus. 0 (0). 1988. 97-116 --	1-6
A	Biological Abstracts/RRM, BR33:81569, S. McCormick et al.: "Identification of genes specifically expressed in reproductive organs of tomato", see the abstract, & Biotechnology; Symposium, Davis, California, USA, August 20-22, 1986. XIX+339P. Alan R. Liss, Inc.: New York, New York, USA. Illus. 0 (0). 1987. 255-266 --	1-6
A	Journal of Cellular Biochemistry, Supplement 12C, 1988, UCLA Symposium on the Molecular Basis of Plant Development, 26 March - 2 April 1988, Alan R. Liss, Inc., (New York, US), C.S. Gasser et al.: "Analysis of floral specific genes", page 137, abstract L 021 see the abstract --	1-6
A	Biological Abstracts/RRM, BR36:27773, C.S. Gasser et al.: "Isolation of differentially expressed genes from tomato flowers", see the abstract, & Plant Gene Research: Basic Knowledge and Application: Temporal and Spatial Regulation of Plant Genes. XIII+344P. Springer-Verlag: Vienna, Austria; New York, New York, USA. Illus. 0 (0). 1988. 83-96 --	1-6
A	Chemical Abstracts, volume 106, 1987, (Columbus, Ohio, US), J.R. Stinson et al.: "Genes expressed in the male gametophyte of flowering plants and their isolation", see page 175, abstract 150569p, & Plant Physiol. 1987, 83(2), 442-7 -----	1-6